



TITLE:

A new Comparative Method of the Pharmacokinetics of the Two Antibiotics by HPLC Analysis after Simultaneous Administration

AUTHOR(S):

HUANG, WU-FANG

CITATION:

HUANG, WU-FANG. A new Comparative Method of the Pharmacokinetics of the Two Antibiotics by HPLC Analysis after Simultaneous Administration. 日本外科宝函 1983, 52(3): 273-298

ISSUE DATE:

1983-05-01

URL:

<http://hdl.handle.net/2433/208863>

RIGHT:

原 著

A New Comparative Method of the Pharmacokinetics of the Two Antibiotics by HPLC Analysis after Simultaneous Administration

WU-FANG HUANG

Second Department of Surgery, Faculty of Medicine, Kyoto University

(Director: Prof. Dr. YORINORI HIKASA)

Received for Publication, March 1, 1983.

Introduction

In the treatment of biliary tract infections with systemic administration of antibiotics, we must consider the following factors; (1) the physical characteristics or age of patients, (2) the renal or hepatic function of patients, (3) sufficiently high biliary excretion of antibiotics to be effective against the infected organisms, (4) lower toxicity of antibiotics to the liver and kidney. To administer safely and effectively antibiotics in keeping with these points, the physician needs to measure drug concentrations in the biological fluids in order to monitor the administration program. This is especially important in the treatment of patients with impaired hepatic and/or renal function resulting in the altered rate of the metabolism of drugs. Therefore, the rapid and accurate monitoring of antibiotic concentration in the human biological fluids is essential in the clinical laboratories.

Several methods are currently available for the quantitative analysis of antibiotics in the biological fluids, such as microbiological and chemical assays including fluorimetric, radio-enzymatic, or radioimmunoassay, and gas chromatography. The microbiological assay has been the most commonly used method to determine the antibiotic concentration in the biological fluids, because it is the most economical and a large amount of samples can be incubated simultaneously^{1,2)}. However, this assay system suffers from various disadvantages, for instances, it is time consuming usually requiring 24 hours to obtain the desired information, and the methodology is not uniform because different test organisms and different media are used for the same antibiotics. Furthermore, the presence of another antibiotic in the sample may often interfere with the assay.

Recently, high performance liquid chromatography (HPLC) as an improved form of liquid

Key words: Cephalosporin, Cephamycin, HPLC, Simultaneous administration, Biliary excretion, Tissue concentration.

索引語: セファロスポリン, セファマイシン, 高速液体クロマトグラフィー, 同時投与比較試験, 胆汁中移行, 組織内濃度.

Present address: Second Department of Surgery, Faculty of Medicine, Kyoto University. 54 Kawara-cho, Shogoin, Sakyo-ku, Kyoto, 606, Japan.

chromatography has been implemented for quantitative assay of antibiotics since the first application of cephalothin and deacetylcephalothin in human serum and urine by COOPER et al. in 1973¹⁶⁾. Thereafter, several papers were presented on the quantitative measurement of penicillins and cephalosporins in serum or urine using HPLC^{7,15,25,30,36,47,48,50,51,61,66,84,85,89)}. The advantages of the HPLC method are rapid analysis, relatively simple preparation of the sample, and highly accurate results.

Clinically, the crossover method has been used for comparison of the biliary excretion of the same class of antibiotics in the same patient. However, it can not completely exclude the inter- and intra-individual factors such as differences in the liver function or in the excretion rate of bile, or the interruption of the enterohepatic circulation of bile acids when the bile samples were collected. Thus, it is generally difficult to assess pharmacokinetic differences when two drugs were given successively to the same patient.

These difficulties do not arise if the drugs to be compared are administered simultaneously. With the aid of a newly elaborated HPLC method^{28,29)}, it is possible to assay two or more antibiotics even if they are simultaneously present in the biological fluids. In this paper a method of simultaneous determination was established, and the biliary excretion was studied by the administration of two cephalosporins or cephamycins to rats and patients with T-tube drain. This method may have significant advantages over the conventional study, and it would appear to be adaptable for comparison of the pharmacokinetics of many cephalosporins and cephamycins.

Materials and Methods

Materials

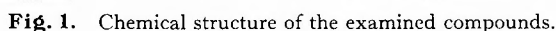
Ceftizoxime (CZX, Fujisawa Pharmaceutical, Osaka, Japan), ceftazidime (CAZ, Glaxo Laboratory, England), latamoxef (LMOX, Shionogi Pharmaceutical, Osaka, Japan), MT-141 (Meijiiseika, Tokyo, Japan), cefotaxime (CTX) and deacetyl-cefotaxime (Hoecht, Tokyo, Japan) were used as supplied (Fig. 1). All solvents and chemicals used throughout this procedure were of analytical grade.

HPLC Assay and Chromatographic Conditions

Chromatography was carried out on an HPLC apparatus (Shimadzu, LC-4A, Kyoto, Japan) equipped with an ultraviolet detector (Shimadzu SPA-2A), connected to a computing integrator (Shimadzu Chromatopac, CR-2A). The detailed HPLC conditions are summarized in Table 1. A precolumn protected the analytic column (4.5 × 4 mm I.D. permaphase ODS) from biological contamination. The chart speed was maintained at 2.5 mm/min in all analyses. The chromatographic peaks were recorded and their representative areas were integrated with a chromatopac integrator. Quantitation was based on integration of the peak areas previously calibrated with known concentrations of the antibiotic.

Microbiological Assays

In addition to the HPLC method, a part of the bile samples were assayed by a microbiological method. The CAZ concentrations were assayed by the paper disc method with the use of *Proteus mirabilis* ATCC 21100 as the test organism. The CZX concentrations in bile were assayed also



Samples of Experimental Animals

Table 1. HPLC Conditions for Simultaneous Determination of Two or More New Cephems in Biological Samples

*phosphate buffer solution ($\text{NaH}_2\text{PO}_4 - \text{H}_3\text{PO}_4$)

groups. The rats were anesthetized with ether, the abdomen was open and the common bile duct was cannulated with a polyethylene tube. The cannula was brought to the exterior and the abdomen was closed. Then the anesthetized rats were placed in a *Bollman* cage for fixation. the animals regained consciousness, the antibiotic was administrated through the tail vein; liver tissue and blood samples were taken at 20, 50, and 80 min after the administration. Because of the limited amount of bile excretion, we collected the bile at the intervals: 0–20, 21–50, and 51–80 min after the administration. Blood samples were collected from the femoral artery after animals were sacrificed at the above indicated time. The liver was removed and washed with distilled water and cleaned with sterile gauge. The liver tissue was weighed and an equal amount of 0.1 M phosphate buffer solution (pH 7.0) was added for homogenization. At each time, the concentrations of serum, bile and liver tissue were calculated from the mean values for 3 animals.

Samples of Clinical Studies

Table 2 summarizes the clinical characteristics of the patients. Case 1 received a simultaneous intravenous (iv) injection of both 0.5 g of CZX and CAZ, and a single iv injection of 0.5 g of CAZ. Serial blood samples were taken from the contralateral ante-cubital vein. Cases 2 and 3 were studied for comparison of the biliary excretion of CZX and CAZ after iv injection of 1 g of both drugs by the crossover method. Case 4 was studied for comparison of the biliary excretion of CZX and CAZ after simultaneous iv injection of 0.5 g of both drugs on the 6th post-operative day when jaundice was still present and on the 30th postoperative day when jaundice was not seen. Case 5 was studied for the biliary excretion of MT-141 and LMOX after simultaneously iv injection of 1 g of both drug and after a single MT-141 1 g injection. Case 6 was studied for two single iv injections (MT-141 1 g, LMOX 1 g) and simultaneous iv injection (MT-141 1 g plus LMOX 1 g). The concentrations of total bilirubins, total bile acids, calcium, and bile flow of each bile sample was also calculated.

All patients except Case 1 had a T-tube inserted in their common bile ducts; the liver or renal function was within normal limits except in Case 4. Bile samples were collected according to the following schedule: a control sample before injection of the drug, then 0–0.5 h, 0.5–1 h, 1–1.5 h, 1.5–2 h, 2–2.5 h, 2.5–3 h, 3–3.5 h, 3.5–4 h, 4–5 h, and 5–6 h after injection. Bile samples were

Table 2. Clinical Characteristics of the Examined Patients

Case	Name	Sex	Age	Weight (kg)	Diagnosis	Antibiotics
1	K. G	M	32	70	Cholelithiasis	CAZ 0.5g plus CZX 0.5g CAZ 0.5g alone
2	O. K	F	71	47	Choledocholithiasis	CAZ 1g CZX 1g (Crossover)
3	T. O	M	55	55	Choledocholithiasis	CAZ 1g CZX 1g (Crossover)
4	S. M	M	82	48	Choledocholithiasis	CAZ 0.5g plus CZX 0.5g Two administrations
5	K. M	F	73	50	Choledocholithiasis	MT-141 1g plus LMOX 1g MT-141 1g alone
6	Y. Y	F	72	50	Choledocholithiasis	MT-141 1g alone LMOX 1g alone MT-141 1g plus LMOX 1g

immediately diluted in phosphate buffer solution (pH 6.0) and injected into HPLC as soon as possible. All samples obtained in this experiment were determined by our newly developed HPLC method except in Cases 2 and 3. Blood samples were coagulated and the sera were separated by centrifugation and immediately extracted with 6% Trichloroacetic acid (TCA).

Preparation of the Standard Solution

Aqueous solutions of two cephalosporins or two cephamycins were prepared in phosphate buffer solution (pH 6.0) to a concentration of 1,000 $\mu\text{g/ml}$ and further diluted with phosphate buffer solutions. An aliquot of 0.1 ml of each of the standard solutions was added to 0.9 ml of rat serum, bile, and liver homogenate, or human serum, and bile to make the 5 standard solutions in the range of 1 to 100 $\mu\text{g/ml}$, these were then incubated at ambient temperature for at least 20 min. Blank samples were prepared in the same manner except for omission of the antibiotics. Standard curves were constructed by plotting the peak area of each cephalosporin or cephamycin against the concentrations. Latamoxef was calculated as the total amount of R-epimer plus S-epimer. The regression line obtained in all cases had a correlation coefficient better than $r=0.999$. The standard solution was freshly prepared each day before chromatographic assay.

Preparation of Sample Solutions

a) In rats: The liver homogenates (1:1 w/v) were prepared in phosphate buffer solution (pH 7.0) using a homogenizer. Equal volumes of 6% TCA were added to the blank, standard, experiment serum or liver homogenate for deproteinization, then stirred rapidly in a vortex-type mixer for 10 sec, placed in an ice bath for 10 min, and centrifugated at 3,200 rpm for 5 min. The resulting supernatant of each sample was transferred from the test tube to another test tube with a *Pasteur* pipette and pass through a 0.22 μm microfilter (Millipore, USA) and immediately injected onto a reverse phase HPLC column. The injection volume was 50 to 100 μl .

However, the bile samples were diluted with phosphate buffer solution (pH 6.0) and thus the deproteinized process before chromatography was unnecessary; the injection volume was 50 μl .

b) In human: Each serum sample (0.5 ml) was mixed with 4.5 ml of 6% TCA. This mixture was kept in an ice bath for 10 min and centrifugation at 3,200 rpm for 5 min. The resulting supernatant was removed from the test tube and passed through a 0.22 μm microfilter. Finally, 25 μl aliquots were injected onto the HPLC column. The preparation of human bile samples was similar to those in rats.

Results

Chromatography of Cephalosporins and Cephamycins

Figure 2 shows representative chromatographic tracings resulting from HPLC of the samples prepared from the extract of liver homogenate of blank samples and these at 20 min after injection, blank bile and bile sample at 20 min after simultaneous iv administration of CZX and CAZ in rats. The peaks of CZX and CAZ were completely separated from each other and not affected by other serum or bile components in these samples. The appearance of the glucuronide metabolite of CZX was clearly observed in rat bile, but was not detected in the human bile sample.

As shown in Figs. 3a and b the peaks of CZX and CAZ in human bile were identical to the

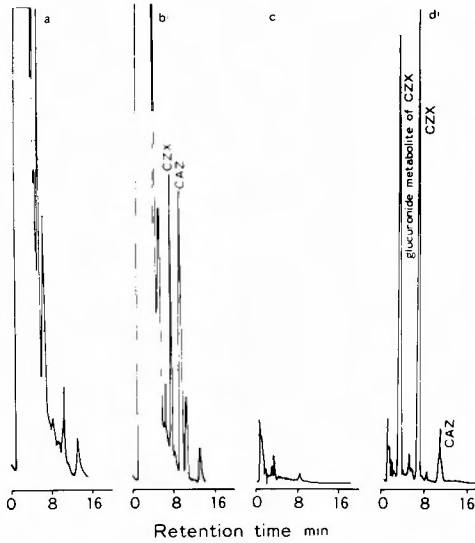


Fig. 2. Chromatograms of (a) extract of blank liver, (b) liver homogenate at 20 min after drug administration, (c) blank bile and (d) bile sample at 20 min after drug administration in rats.

results described in Fig. 2. HPLC in Fig. 3c showed the complete separation of MT-141, deacetyl CTX, LMOX and CTX in human bile. In addition, the resolution of R- and S-epimers of LMOX was noted.

Figures 4 and 5 show the representative standard curves obtained from the rat's liver homogenate and human bile containing various amounts of CZX and CAZ. There was a good linear relationship between the peak area and the concentration of CZX and CAZ from 0 to 20 $\mu\text{g/g}$ in

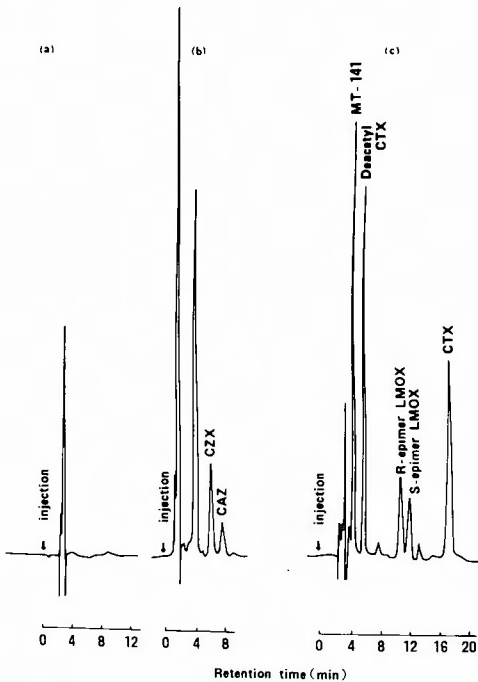


Fig. 3. Chromatograms of (a) human bile blank (pre-injection), (b) human T-tube bile at 120 min after simultaneous administration of 0.5 g of CAZ and 0.5 g of CZX, (c) bile sample of human spiked with MT-141 deacetyl-CTX, CTX, and LMOX.

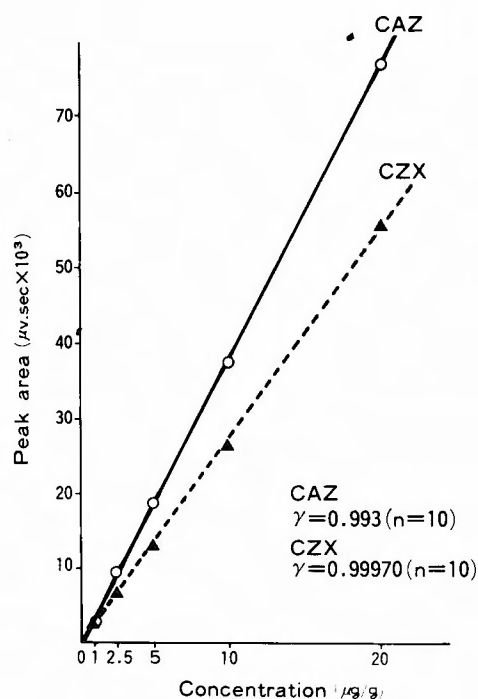


Fig. 4. Standard curves for simultaneous determination of CAZ and CZX in the liver homogenate of rats.

rat's homogenate and from 0 to 100 $\mu\text{g}/\text{ml}$ in human bile; each of these had a correlation coefficient greater than $\gamma=0.999$. Similar findings were found for other antibiotics or other samples examined in this experiment.

The reproducibility and accuracy of the chromatographic methods are summarized in

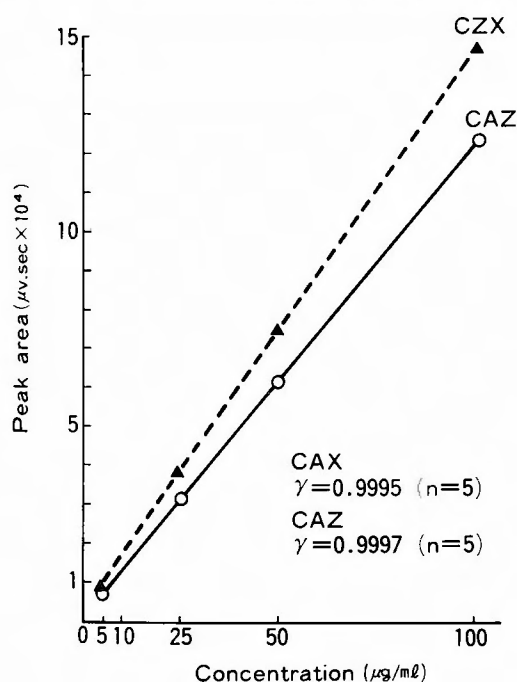


Fig. 5. Standard curves for simultaneous determination of CZX and CAZ in human bile.

Table 3. Reproducibility and Accuracy of Simultaneous Determination in Biological Samples

Sample	Antibiotics	Added conc. $\mu\text{g}/\text{ml}$	Mean% of actual conc.	CV ($n=10$)
Rat serum	CZX	10	90%	0.6%
		20	90	0.9
	CAZ	10	94	4.0
		20	93	2.0
Rat bile	CZX	25	101	0.6
		50	101	0.7
	CAZ	25	100	0.9
		50	96	1.0
Human bile	CZX	100	93	0.6
	CAZ	100	97	4.3
Human bile	MT-141	25	97	2.5
		50	99	2.3
	LMOX	25	94	4.0
		50	96	2.8

Table 3. The coefficient of variation was less than 5% in all analyses indicating that these HPLC methods were satisfactory for antibiotic determination.

The limit of detection in the rat's liver was as low as 0.5 $\mu\text{g}/\text{g}$ and 0.5–1.0 $\mu\text{g}/\text{ml}$ for most biological samples.

As shown in Table 4, the recovery for CZX and CAZ from human serum was 97.5 and 93.5%, respectively, indicating that the deproteinized process with 6% TCA was suitable for CZX and CAZ.

Pharmacokinetics of CZX and CAZ in Rat

The serum levels of CZX and CAZ after the simultaneous iv administration and single iv administration are shown in Table 5. The serum level of each drug in the simultaneous iv administration at 20, 50, and 80 min after injection was slightly lower than those in the single iv administration. In particular, the level of CZX at 20 min after simultaneous administration were significantly lower ($p < 0.05$). Moreover, the biological half-life of both drugs in the simultaneous iv administration was slightly shorter than those in the single iv administration.

Table 4. Analytic Recovery of Cefizoxime (CZX) and Cefazidime (CAZ) in Serum

Concentration added to serum ($\mu\text{g}/\text{ml}$)	Concentration measured*	Recovery (%)
1	CZX : 0.944	94.4%
	CAZ : 0.9	90 %
10	CZX : 10.2	102 %
	CAZ : 10.0	100 %
50	CZX : 48.1	96.2%
	CAZ : 45.7	91.3%
Mean		CZX : 97.5% CAZ : 93.7%

* Each value represents the mean of duplicate analyses

Table 5. Serum Levels of CZX and CAZ in Rats

		(μg/mL HPLC)			
		20min	50min	80min	T _{1/2}
CZX	CZX ⁺	24.2±1.8	15.0±6.3	4.3±0.6	17min
	CZX*	42.7±1.6	17.1±6.1	8.1±1.5	24min
CAZ	CAZ ⁺	34.0±2.5	22.5±6.2	7.7±0.6	18min
	CAZ*	42.8±0.7	27.2±6.4	13.4	29min

Mean ± SE

+ Simultaneous iv administration group (CAZ 20mg/kg plus CZX 20mg/kg).

* Single iv administration group (20mg/kg for each drug).

Figure 6 depicts the hepatic concentration time curve of CZX and CAZ in the simultaneous iv administration. The hepatic concentrations at 20, 50 and 80 min after injection were 13.6 ± 4.5 , 1.8 ± 1.0 and less than $0.5 \mu\text{g/g}$ (below the detectable limit), respectively for CZX, and 5.0 ± 0.2 , 3.4 ± 0.1 and $1.8 \pm 0.1 \mu\text{g/g}$, respectively for CAZ. The disappearance of CZX from liver tissue was faster than that of CAZ.

The hepatic concentration of CZX and CAZ was compared between the simultaneous and the single administration, and their data are shown in Table 6. In order to correct the individual variation of the hepatic blood flow among the rats, we measured the hepatic serum ratio at each time for each antibiotic; no significant difference between the single and the simultaneous administration at each time for both antibiotics was seen (Table 7).

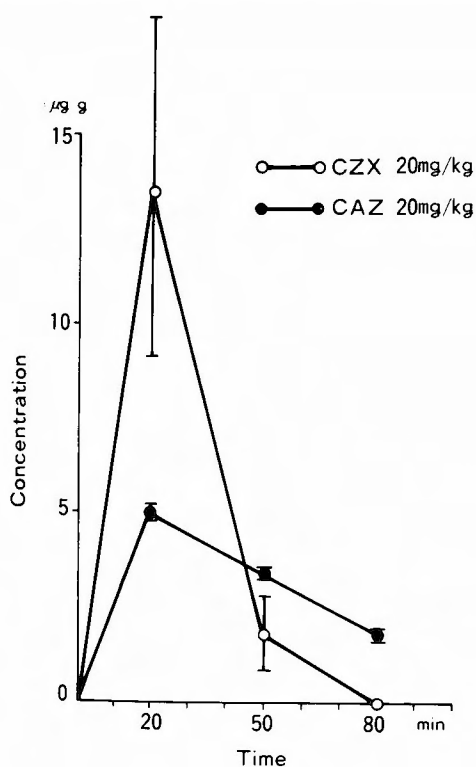
**Fig. 6.** Tissue concentration of CZX and CAZ in the liver of rats.

Table 6. Tissue Concentrations of CZX and CAZ in the Liver of Rats

		(μg/g, HPLC)		
		20min	50min	80min
CZX	CZX ⁺	13.6±4.5	1.8±1.0	<0.5
	CZX*	22.6±7.8	1.5±0.3	0.53±0.26
CAZ	CAZ ⁺	5.0±0.2	3.4±0.1	1.8±0.1
	CAZ*	6.3±0.3	5.6±1.0	4.9±0.4
		Mean ± SE		
		+ Simultaneous iv administration group (CAZ 20mg/kg plus CZX 20mg/kg)		
		* Single iv administration group (20mg/kg for each drug)		

Table 7. The Relative Ratio of Hepatic Tissue Concentrations to Serum Levels of CZX and CAZ in Rats

		(%)		
		20min	50min	80min
CZX	CZX ⁺	59.6±37.3	11.3±3.1	N.D.
	CZX*	54.0±33.9	13.2±0.6	7.2±7.1
CAZ	CAZ ⁺	15.8±1.9	17.9±9.1	23.8±4.2
	CAZ*	14.8±1.7	21.6±3.2	20.5±8.1
		Mean ± SD		
		+ Simultaneous iv administration group (CAZ 20mg/kg plus CZX 20mg/kg)		
		* Single iv administration group (20mg/kg for each drug)		

The biliary excretions of both drugs in the rat are shown in Fig. 7 and Table 8. The black line and dotted line indicate the simultaneous and the single administration, respectively. Although the biliary excretion of each antibiotic in the single administration was slightly higher than those in the simultaneous administration, there was no significant difference between the single and simultaneous administration. The CZX level reached a peak at 0–20 min after injection, in contrast, the peak level of CAZ was found at 21–50 min after injection. The peak level of CZX was much higher than CAZ (637±98 μg/ml, versus 46.2±8.8 μg/ml, respectively). Table 9 shows the fractional and total bile recovery of each antibiotic for the single and simultaneous administration. The total recovery of CZX was about 10 times higher than CAZ (2.08 versus 0.21%), and there was also no significant difference between the two administrated groups. Thus, the biliary excretion of CZX and CAZ in the doses given are independent of each other after simultaneous iv administration to rats.

Pharmacokinetics of Antibiotics in Patients with Biliary Tract Diseases

The successful experimental studies in rats, suggested that this methodology could be applied

Table 8. Concentration of CZX and CAZ in Rat Bile

		(μg/ml, HPLC)		
		0–20min	21–50min	51–80min
CZX	CZX ⁺	637±98	387±63	80.1±15.4
	CZX*	629±82	393±28	104±2.4
CAZ	CAZ ⁺	37.9±6.4	46.2±8.8	20.7±5.5
	CAZ*	47.8±3.7	63.8±6.4	48.6±5.0
		Mean ± SE		
		+ Simultaneous iv administration group (CAZ 20mg/kg plus CZX 20mg/kg)		
		* Single iv administration group (20mg/kg for each drug)		

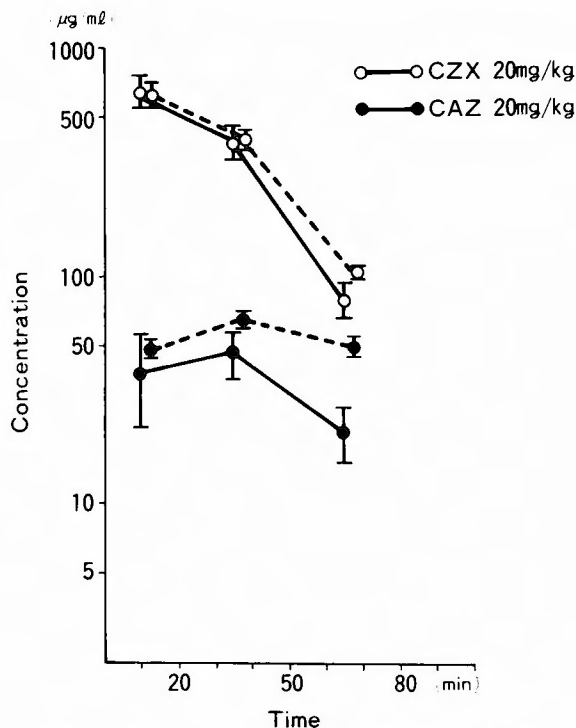


Fig. 7. Concentration of CZX and CAZ in Rat Bile.

to human.

a) Simultaneous administration

In Case 1, the simultaneous iv administration of 0.5 g of CZX and CAZ was performed. The serum concentration time curves are shown on the left side of Fig. 8. The serum concentrations of both CAZ and CZX showed a nearly similar pattern at all times. On the right, the serum concentration time curves of CAZ after the single iv administration of 0.5 g of CAZ and the simultaneous iv administration of 0.5 g each of CAZ and CZX are shown. The CAZ concentration was not affected by the simultaneous administration with CZX compared with the single administration of CAZ.

Cases 2 and 3 were studied for the biliary excretion of CAZ and CZX after 1 g iv injection

Table 9. Biliary Recovery of CZX and CAZ in Rats
(% of dose, HPLC)

		0-20min	21-50min	51-80min	Total
CZX	CZX	0.99±0.57	0.95±0.38	0.14±0.06	2.08
	CZX*	0.89±0.49	0.87±0.53	0.12±0.07	1.88
CAZ	CAZ	0.04±0.05	0.11±0.05	0.04±0.03	0.21
	CAZ*	0.08±0.02	0.11±0.04	0.08±0.01	0.27

Mean ± SD

+ Simultaneous iv administration group (CAZ 20mg/kg plus CZX 20mg/kg)

* Single iv administration group (20mg/kg for each drug)

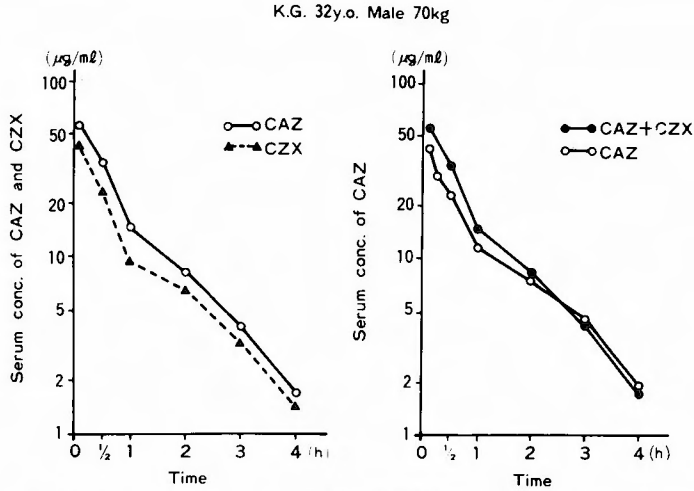


Fig. 8. Serum concentration of CAZ and CZX after single or simultaneous iv administration of 0.5 g of each drug.

by the crossover method, the peak level of CZX was reached at 30–60 min after injection and thereafter, rapidly decreased with the time, as shown in Figs. 9 and 10. On the contrary, CAZ concentration required a much longer time to reach the peak level, with a slower disappearance from bile. Finally, peculiar patterns were seen in the biliary excretion between both antibiotics, despite the fact that both antibiotics were mainly eliminated by the renal route and had similar serum half-life.

b) Cephalosporins

In such a situation, the simultaneous administration of two drugs is absolutely necessary in order to confirm this discrepancy.

In Case 4, two simultaneous administrations of 0.5 g each of CZX and CAZ were performed.

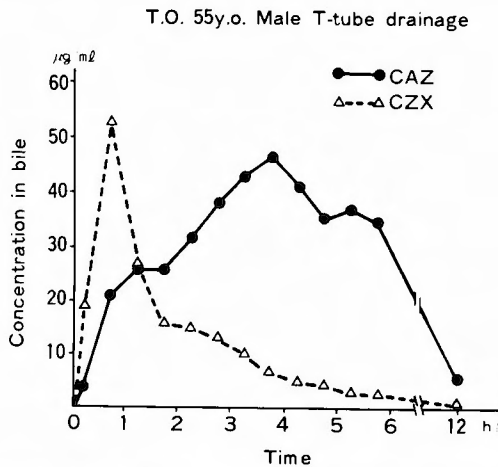


Fig. 9. Biliary excretion of CAZ and CZX after 1 g iv administration by crossover method.

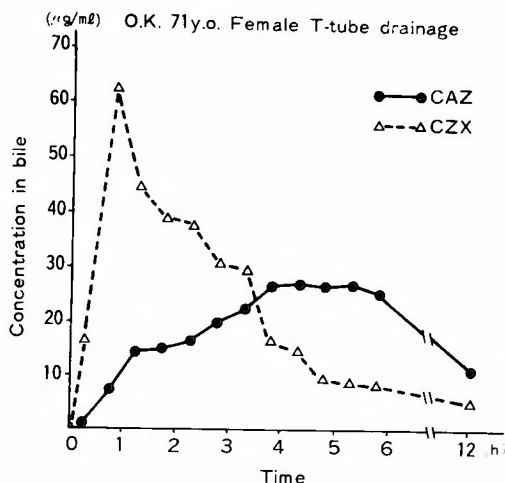


Fig. 10. Biliary excretion of CAZ and CZX after 1g iv administration by crossover method.

The first simultaneous administration was performed on the 6th postoperative day after T-tube drainage, when abnormal liver function was still present (GOT 36, GPT 28, ALP 120 mU/ml, T. bilirubin 3.2 mg/dl), the biliary excretion of both drugs were followed by HPLC as shown in Fig. 11. Similar excretory patterns of both drugs were observed, unlike the previous cases studied by the crossover method. However, on the 30th postoperative day, when the liver function had returned to normal and the enterohepatic circulation of bile acids had returned to the physiological state, the second simultaneous administration was performed. The biliary excretion at that time is presented in Fig. 12. Different patterns of biliary excretion for both drugs were obtained compared with the first administration, producing a pattern nearly identical with the one noted in Cases 2 and 3 by the crossover method, or with the excretory pattern in rats.

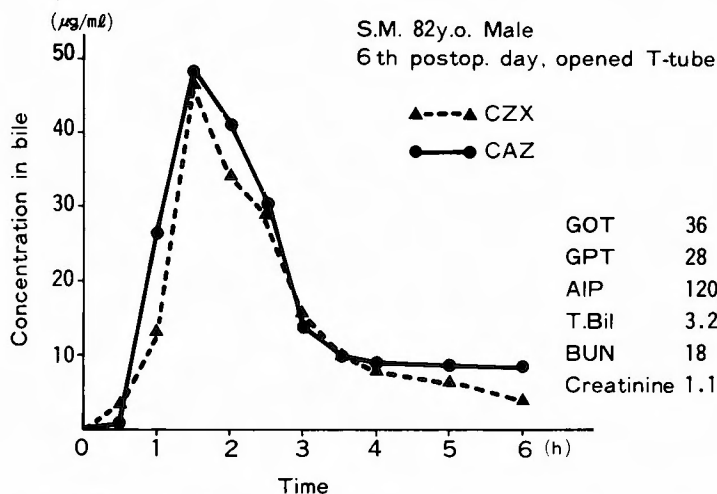


Fig. 11. Biliary excretion of CAZ and CZX after simultaneous iv administration of 0.5 g of both drugs.

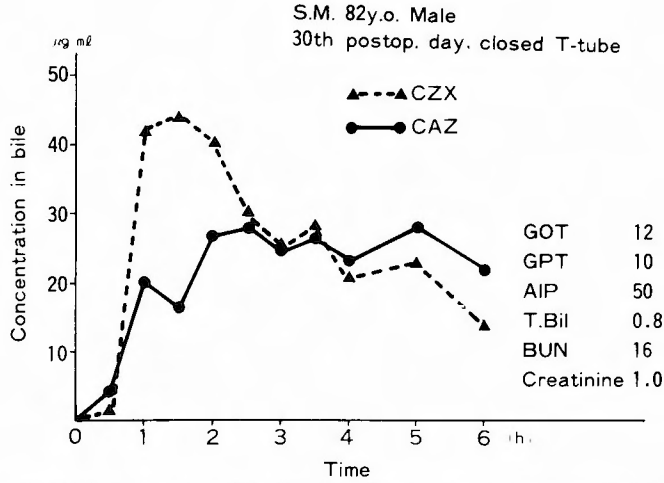


Fig. 12. Biliary excretion of CAZ and CZX after simultaneous iv administration of 0.5 g of both drugs.

c) Cephamycins

An attempt was made to determine two new cephamycins, LMOX and MT-141, simultaneously in human bile; the HPLC conditions are given in Table 1.

In Case 5, 1 g each of LOMX and MT-141 was simultaneously administered on the 21th postoperative day. The biliary excretion of both drugs are shown in Fig. 13a. The peak biliary concentration for each antibiotic appeared at 2h after the simultaneous administration. At all

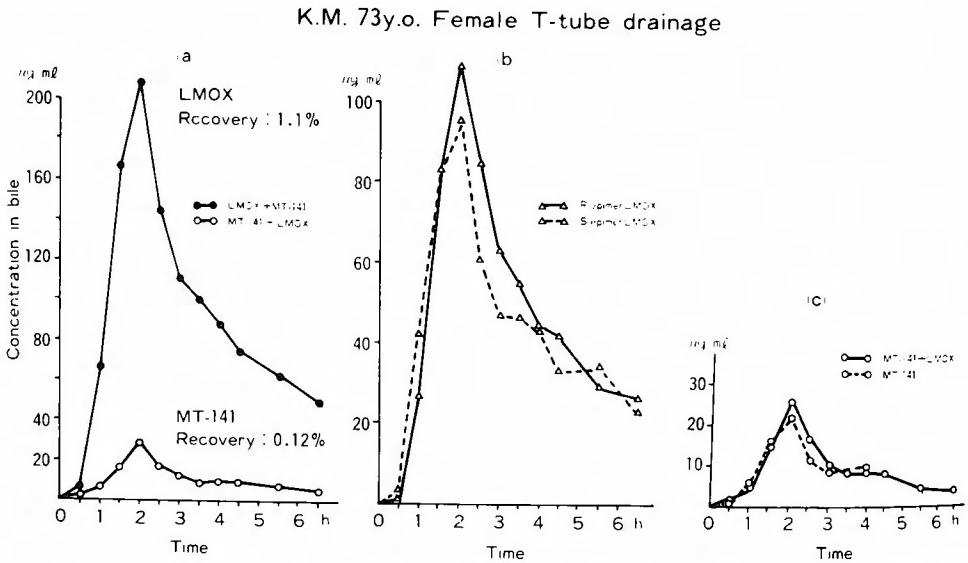


Fig. 13. Biliary excretion of LOMX and MT-141; (a) bile concentration time curves after simultaneous administration of 1 g of LMOX and MT-141. (b) R- and S-isomers after simultaneous administration of 1 g of LMOX and MT-141, (c) bile concentrations of MT-141 after 1 g of single administration and simultaneous administration of 1 g of MT-141 and 1 g of LMOX.

time intervals, LMOX reached much higher levels than those for MT-141, with the peak level of 209.3 $\mu\text{g/ml}$ for LMOX (R- plus S-epimer) and 28 $\mu\text{g/ml}$ for MT-141. The total biliary recoveries of LMOX and MT-141 were 1.1% and 0.12% of the dose, respectively. Figure 13b shows the serial changes of R- and S-epimer of LMOX after the simultaneous administration. Both epimers were excreted from the bile with a similar pattern. Figure 13c shows the MT-141 concentration in bile after the single 1 g iv administration and after the simultaneous administration with 1 g of LMOX in Case 5. The peak level of MT-141 after the single administration was slightly lower than that in the simultaneous administration, but no great difference was found at others time intervals.

Factors Influencing Biliary Excretion

Three antibiotic administrations were performed in Case 6. The first administration was performed on the 15th postoperative day, with 1 g of MT-141 iv injection. The 2nd administration was performed on the 16th postoperative day, with 1 g of LMOX iv injection. The 3rd administration was performed on the 20th postoperative day, with 1 g each of MT-141 and LMOX simultaneous iv injection. The antibiotic concentration in bile, mean value of total bilirubin, total bile acids, calcium, and the bile flow rate are listed in Table 10.

The biliary concentration for each antibiotic after the simultaneous administration was lower than those with the single administration. In addition, the bile flow rate during the 3rd administration was much larger than that obtained during the 1st and 2nd administration, and the concentration of antibiotics, mean value of total bilirubin, total bile acids, and calcium were also reduced by an excessive amount of the bile. As shown in Table 10, the total biliary recovery for each drug in the single or simultaneous administration remained constant. It is speculated that the same biliary amount of each antibiotic was excreted after the single or simultaneous administration in spite of the marked difference of the bile flow rate on the different testing time. The excreted amount per 30 min in bile is represented in Fig. 14. The concentration of total bilirubin, total bile acids, and calcium were also measured for each time interval; these showed no significant

Table 10. Biliary Excretion of MT-141, LMOX, Total Bilirubin, Total Bile Acids, Calcium and Bile Flow Rate After Single Administration For Each Drug and Simultaneous Administration for Both Drugs in Case 6

		Antibiotic concentration in bile ($\mu\text{ g/ml}$)										Bile constituents				Total recovery for 6 hours
		30'	1	1 30'	2	2 30'	3	4	5	6	total bilirubin (mg dL)	bile acids (mM, L)	bile flow rate (mL min)	calcium (meq L)		
MT-141 1g		0.6	5.5	19.5	16.4	19.7	9.2	9.0	2.6	2.0	14.7	8.5	0.54	3.6	0.15%	
LMOX 1g		2.0	22.0	26.0	31.6	24.7	20.2	13.3	11.8	7.0	30.2	12.5	0.32	3.4	0.17%	
MT-141 1g plus LMOX 1g	MT-141	1.0	3.0	9.6	7.5	9.0	3.5	4.2	2.5	0.6	10.2	3.2	1.1	3.0	MT-141 0.15%	
	LMOX	2.0	10.5	10.7	8.0	6.0	3.0	4.5	3.0	4.2					LMOX 0.18%	

Y. Y. 72 y.o. Female 50kg T-tube drainage

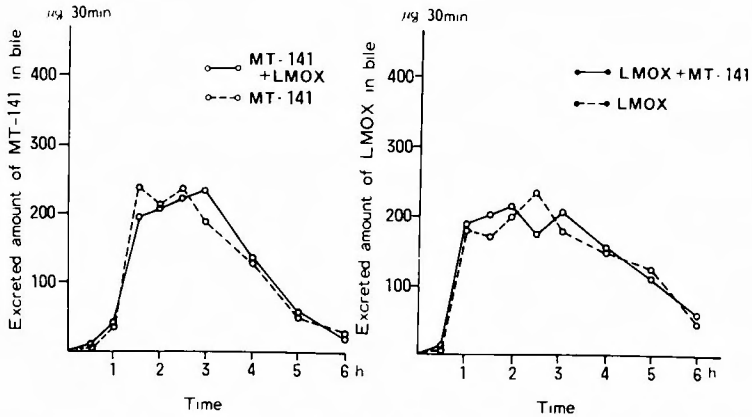


Fig. 14. Biliary excretion of MT-141 and LMOX per 30 min when given alone compared with simultaneous administration of 1.0 g of each drug.

variation after administration of both drugs with single or simultaneous administration.

Discussion

Clinical assays have been developed for some antibiotics, for example, fluorimetric procedure for tetracyclines⁴⁰⁾, erythromycin⁷⁷⁾, cephaloridine⁷⁸⁾ and cefuroxime⁵⁾, and spectrophometric assays for chloramphenicol³¹⁾ and erythromycin¹⁹⁾. These procedures employed complicated extraction and lack specificity. Radioimmunoassay has been used for gentamicin¹²⁾, sisomicin¹³⁾ and clidamycin²¹⁾, and radioenzymatic assays for gentamicin, tobramycin, amikacin²³⁾. These assay systems are technically quite complicated and require specially trained personal to handle the radioisotopes. Polarographic method for the determination of cephaloridine, cephalothin, and cephalexin in an ultrafiltrate of serum was reported by BENNER¹⁰⁾; cephalosporins do not lend themselves to simple gas chromatography because of their low volatility. All chemical assays described above suffer from restricted applicability and are not easily adaptable to a wide range of antimicrobial agents.

COOPER et al.¹⁶⁾ reported an assay for cephalosporin using the anion exchange chromatography for the determination and separation of cephalothin and deacetyl cephalothin from human serum. BUHS et al.¹⁴⁾ also described a method using HPLC with the anion exchange mode to separate cephalothin, cefoxitin and their deacetylated metabolites in human urine. Subsequent methods have used the simple protein precipitation or the direct injection of the biological samples using reverse phase HPLC^{6, 51, 90)}

WHITE et al.^{79, 80, 81)} reported the separation and determination of cephalosporins, penicillins, tetracyclines and chloramphenicol by the reverse phase HPLC, and described that the reverse phase mode had several advantages over the ion exchange mode: namely ① mobile phases are inexpensive and simple, ② stationary phase (column) are permanently bonded (ODS or C₁₈

packing) and stable over the wide range of pH (2.0 to 7.8) and temperature, ③ longer life of column, ④ retention times are easily controlled by changing the composition of the organic solvent, ⑤ the wide range of antibiotics can be separated on a single column by suitably changing the composition of the mobile phase, ⑥ the separation can be carried out at ambient temperature.

The modern HPLC⁽⁸¹⁾ is equipped with a small diameter column packed with small particles (5 μm) to increase the column efficiency. Therefore, it has several advantages when compared with the large particle, efficiency pellicular column: ① sharper peak ② good separation ③ shorter analysis times ④ large sample size ⑤ high sensitivity. Sometime, an ion pair agent, such as tetrabutyl-ammonium hydrogen sulphate, was added to the mobile phase to permit the separation of the most polar compounds which were difficult to separate on the reverse phase mode⁽³⁵⁾.

In recent years there were many reports on the measurement of antibiotics in the biological fluids by HPLC, and the determination of cephalosporins in human serum or urine has been reviewed by ANHALT⁽⁶⁾, and YOSHIKAWA⁽⁹⁰⁾. The published data on the application of HPLC for the analysis of antibiotics in bile or the liver tissue are scarce.

In the 1980's remarkable advances have been made in the development of cephalosporins and cephamycins. The HPLC is a very useful apparatus for the pharmacokinetic study of new drugs. In our laboratory, we have so far studied the excretion of various drugs, such as miloxacin (MLX)⁽⁶⁸⁾, cefotetan (CTT)⁽⁷²⁾, cefpiramide (CPM)⁽⁷³⁾, cefbuperazone (CBPZ)⁽⁷⁴⁾, and ceftriaxone (CTRX)⁽⁷⁶⁾ in bile by HPLC. Not only the parent compound but also the glucuronide conjugate (MLX), metabolite (CBPZ) or the tautomer (CTT) were measured in the bile.

The procedures used in this study did not incorporate any internal standard. Therefore, the results might have been affected by such variables as extraction or dilution. Instead of an internal standard, the standard curve was constructed each day before sampling, and its good linearity was confirmed. The recovery rates with serum or bile in rats and patients, however, demonstrated a high accuracy and thus, these methods may be applicable for pharmacokinetic study in experimental animals and clinical cases.

Although the β -lactam antibiotics have all been detected by ultraviolet absorption at 254 nm in the literature^(6,49,79,80,81,90), the 280 nm was chosen for determination of most samples in this experiment, because the blank (background) peaks were small at that absorption, and it lessened the possibility of multiple components in a single peak. Since the major problem had been one of detection⁽⁶⁾, the lowest limit of detection in this experiment was adequate for most of the clinical assays of antibiotics. When the sensitivity was set at the maximum of 0.005, the injection size was 50 to 100 μl and all samples could be detectable as low as 0.5 to 1 $\mu\text{g/ml}$.

In the preliminary stage of this experiment, the author used the combination of acetic acid ammonium-methanol, or water-methanol as the mobile phase and the separation was incomplete. When a combination of sodium dihydrogen phosphate-acetonitrile was used as a mobile phase, the baseline resolution of compounds could be achieved. It was found that the phosphate buffer solution gave shaper peaks and a better separation. With this method, all samples were analyzed within 20 min. Therefore, the gradient elution system was not applied, since the change in the equilibrium time from 30 to 60 min was required for the column. Repeated injec-

tions of samples might increase the inlet pressure of the column. Therefore, the column must be eluted overnight with a large amount of distilled water and acetonitril (v/v : 40/60) to prevent the obstruction of column.

One of the advantages of the present method is simple pretreatment of bile samples. The 0.22 μm filter was used to remove the visible particles in bile and macromolecules of the serum protein as well.

It is well known that the most common organisms isolated from the biliary tract include *E. coli*, *Klebsilla*, *Enterobacter*, and *S. faecalis*⁷¹⁾. In recent years, there is a high frequency of two or more pathogens isolated from positive bile culture in patients who had undergone biliary tract surgery^{62,71)}. Especially, there is a trend that the frequency of anaerobes as *B. fragilis* from bile increases¹¹⁾. This infection is often resistant to chemotherapy, and is often accompanied by renal and/or hepatic dysfunction.

In the treatment of patients with biliary tract infections, it can be said that the underlying disease should be removed and that the most adequate antibiotic should be chosen and administered⁷⁵⁾. On such occasion, an antibiotic, which has a broad spectrum of antibacterial activity against not only gram-negative bacilli but also anaerobic bacteria, which is stable in β -lactamase, excellent in biliary excretion, and of lower toxicity should be chosen⁷⁵⁾. Therefore, in severe infection such as in aged patients with multiple organs failure⁷¹⁾, we must select two or more antimicrobial agents to combat infected microbes.

On such occasion, carefully monitoring antimicrobial concentration in the biological fluid is absolutely necessary to prevent severe toxicity and adjust the adequate dosage for the next administration³⁷⁾. However, bioassay is not useful for simultaneous determination of multiple drugs in the same sample. HPLC is needed for studying the pharmacokinetics of two or more drugs coadministered in patients with altered drug states.

We have advocated for sometime that the crossover method, which eliminates the effects of various background factors and which makes it possible to compare the concentration of two or more kinds of drugs in bile⁶⁹⁾, may be more useful than by simply obtaining the average concentration of drugs in bile from a T-tube, although studies have been conducted at several institutions on a nationwide basis.

However, even by the crossover method, it is impossible to eliminate discrepancies in the condition resulting from the administration of each drug on the different days. That is, even if the T-tube was closed and the enterohepatic circulation returned to a normal state prior to the implementation of the comparative test by crossover method, the minor effects of bile acids and other bile constituents lost on the first day, occasionally appear on the second day. There is a possibility that a drug the concentration of which is measured on the first day will be slightly advantageous because of its favorable condition. This fact cannot be ignored.

Therefore, two kinds of drugs should be administered at the same time and the concentration of the two drugs should be measured simultaneously by HPLC method, so there is an equivalent to compare the excretion of the two drugs into bile under identical conditions^{28,29)}. This is the best method.

There are few published papers about the simultaneous administration of two cephalosporins for comparison of their pharmacokinetics by HPLC, for example, cefroxadin and cephalixin³⁴⁾, cephalothin and cefazolin⁸⁶⁾, cefazolin and LMOX⁵⁴⁾. However, the comparison of biliary excretion for the same group of antibiotics after simultaneous administration has not been reported.

The author advocates the necessity of simultaneous administration to a patient under the same condition, if the patient's background factors (age, body weight, liver function, bile excretion rate, bile acids concentration, etc) are to be more certainly excluded. Looking at the result of simultaneous administration in Case 4, with and without jaundice despite the fact that the concentrations of antibiotics in the bile and the total recovery generally tended to decrease at the time when patient had jaundice, rather than at the time when he had no jaundice, there was no difference in total recovery of CZX. On the other hand, the concentration of CAZ in the bile reached the peak at 4–5 hours after the administration that took place when he had no jaundice; but it reached the peak at 1–2 hours after the administration that took place when he had jaundice, showing the same pattern of the biliary excretion in CZX. It is suggested from this fact that CAZ excretion into bile is controlled by the total amount of bile acids.

Namely, evidence is provided that the biliary excretion of antibiotics can be influenced by the surgical operation or liver function and occasionally produces different patterns, depending on the times of the estimation. CAZ and CZX have a similar serum protein binding, serum half-life, and both drugs are mainly excreted by the kidney. Though the markedly different patterns of the biliary excretion is unclear, this may be due to the difference of pooling time within the liver cell.

The CAZ concentration in bile increases at the time when the function of the kidney has extremely decreased with the creatinine clearance value being 0–8.3 ml/min, and it reportedly reached 88.4–133.2 $\mu\text{g/ml}$ after 8 hours⁶⁵⁾. Therefore, it can be said that the excretion of CAZ depends upon the balance of function between the liver and the kidney²⁷⁾.

LMOX is a mixture of 52.6% R- and 47.4% S-epimer⁴⁶⁾. The R-epimer is twice as active as S-epimer in vitro against several species of bacteriae⁸³⁾, and they differ in protein binding⁸⁸⁾, 53% for R-epimer, 67% for S-epimer. LUTHY et al.^{38,39)} suggested that the kinetics of the two epimers might differ in adults. NAHADA et al.⁴⁶⁾ also reported different pharmacokinetics of R- and S-epimers in children. Thus, the biliary excretion of LMOX was variable⁴³⁾. On measuring the biliary excretion of LMOX following the simultaneous administration with MT-141 in Cases 5 and 6, the marked variation of biliary concentration of LMOX was also seen between two Cases (Fig. 13, Tab. 10), although the R- and S-epimers of LMOX biliary concentration time curves are almost identical.

Although the biliary concentrations of MT-141 and LMOX during the 3rd administration were much lower compared to its single administration (1st or 2nd administration), the total recovery or excreted amounts per 30 min for each drug in single or simultaneous administration remained constant (Table 10). It is suggested that the large amounts of bile secretion at the 3rd administration might be due to the effect of the excessive secretion of bile acid independent flow

stimulated by secretin or others gastro-intestinal hormones. In such case, a larger dose of antibiotics is needed to achieve the effective antimicrobial level in the bile⁶⁰⁾. The stability of β -lactam in bile is not good even when stored at -80°C , since the antibiotic activity may be decreased by the decomposition of alkaline bile salts⁷⁴⁾, and the epimers of LMOX might interconvert in stored samples⁴⁵⁾. Therefore, all bile samples were immediately examined after collection in this experiment.

The biliary excretion of antibiotics shows marked individual variation, some antibiotics are readily excreted into bile while others not so extensively^{22,43,55,56)}. Although, recently, many papers discuss the mechanism of the biliary excretion of antibiotics, the clear mechanism remains controversial^{4,9,17,41,57,63)}. Several important determinants such as molecular weights, polarity, serum or tissue binding activity or others physico chemical properties have been reported. HIROM et al.²⁶⁾ found that a species variation in the threshold molecular weight factors for the biliary excretion of certain organic anions, 325 ± 50 for rat, 400 ± 50 for guinea pig, and 475 ± 50 for rabbit. RYREFLDT et al.⁵⁹⁾ investigated the relation between polarity and the biliary excretion for 10 types of penicillin in rats and concluded that a relation existed; with increasing polarity in the side chain of the penicillin molecule, biliary excretion increased. LEVINE⁴¹⁾ speculated that a threshold molecular weight of 500 to 600 exists in humans, although lack of extensive investigation is lacking, it is difficult to make such a generalization. WRIGHT et al.⁸⁷⁾ reported the biliary excretion of 18 cephalosporin derivatives with changing 3- or 7-side chain structures in rats, and concluded that the molecular weight is a dominant factor influencing the degree of the biliary excretion, the threshold molecular weight was about 450.

In addition, it is well known that the properties of lipophilicity or other physicochemical characteristics are capable of affecting the biliary excretion⁷⁾. SHIMIZU et al.⁶⁴⁾ examined the ability of the binding of 9 penicillins and 9 cephalosporins to 100,000 G supernatant fluid of human liver homogenates, and concluded that the drugs with lesser binding were mainly excreted by the renal route, in contrast, those with higher binding to the liver homogenate produce lesser amounts of drugs excreted by the kidney. Therefore, the tissue binding of antibiotics may be an important factor in determining the biliary excretion.

Several steps are involved in the hepatobiliary fate of antibiotics, i.e. uptake, binding, metabolism (for some drugs), intracellular translocation, and canalicular transport. The influence of serum protein binding on biliary excretion of drugs is not clear⁸⁾.

ARIAS et al.³²⁾ have described a group of soluble proteins in the rat liver that bind anionic compounds, and designated them as Y-Z proteins. They soon recognized that the Y protein was mainly responsible for the treatment of many compounds and introduced the term "ligandin" to identify the major binding protein. HABIG et al.²⁴⁾ revealed that the hepatic ligandin also bound glutathione and was identical to glutathione transferase B. KORNGUTH et al.³³⁾ examining the ability of rat hepatic ligandin to bind antibiotics demonstrated specific binding of cephalothin, cephalixin, tetracycline, penicillin G, chloramphenicol, and nitrofurantoin, but not erythromycin or polymyxin B. Rat liver ligandin bound these drugs to about the same extent and in the same order as did rat serum albumin. They suggested that ligandin might play an important role in

the hepatic uptake and transport of these drugs from serum.

NISHIYA et al.⁵³⁾ reported that both human anionic GSH S-transferases and human cationic GSH S-transferases have similar binding capacity to the antibiotics (cefotetan, benzylpenicillin, cefazolin, chloramphenicol, and gentamicin) and concluded that the extent of binding of the antibiotics to human liver GSH S-transferases is proportional to the extent of the biliary excretion of these antibiotics suggesting that human liver GSH S-transferases plays an important role in the transport of certain antibiotics from plasma, through liver cells, into canalicular bile.

This methodology would be applicable to critical patients in combination therapy with three or more drugs. The dosage regimens including the injected dose, dosage interval, and duration of administration could be automatically monitored by HPLC in order to ensure the maximal therapeutic effect and reduce the risk of serious toxicity. More recently, a new device, fully automatic HPLC with directly injected samples without sample pretreatment had been developed⁵⁸⁾. For the future, at the bedside rather than in the laboratory the HPLC connected to a computer, like an auto analyzer could be used, and it would permit the clinicians to make rapid monitoring of antibiotic concentrations in biological samples⁴⁴⁾.

Conclusion

The antibiotic concentration in bile has been measured until now by the bioassay method. However, since the pathological condition of a patient usually changes with time, the resulting measurements, are not useful for clinical practice. With consideration given to the difference among patients' background factors, for example, liver dysfunction or jaundice, the author has emphasized that the comparative crossover method of the same patient is more useful than the mere mean values of several cases obtained from the patients with T-tube drain. But even the comparative crossover method has some problems to be solved including the matter of daily changes in the amount of bile excreted.

The author has paid attention into consideration the fact that the HPLC method is useful for the simultaneous determination of the antibiotics in bile samples in which the same class of antibiotic concomitantly exist, though the bioassay method heretofore in use is of no avail for this purpose. The following are the results of this experiment.

- (1) Several HPLC conditions were developed which enabled us to separate and determine two or more cephalosporins or cephamycins that are present in serum, bile and the supernatant of liver homogenate.
- (2) Two kinds of cephalosporins (CZX and CAZ) were intravenously administrated to rats, the results showed that simultaneous separation and determination of the two antibiotics administrated intravenously could be performed, and no competition of biliary excretion occurred between two antibiotics.

Though the two antibiotics show similar concentration curves in serum, different concentration curves of both antibiotics in the hepatic tissue were found. This difference serves as a main factor to cause variations in the concentration patterns in bile.

- (3) The determination of the concentration of the two cephalosporins or cephamycins in serum and bile were conducted in six cases undergoing T-tube drainage. Through this determination, it was proven that even antibiotic which show similar curves of concentration in serum can assume remarkably different concentration patterns in bile.

This result was obtained with involving the enterohepatic circulation for bile constituents including bile acids which cannot be removed in the conventional crossover method on different days and also without being affected by the differences in the amount of excreted bile depending on the different days of bile collection.

The HPLC method developed by the author, that is, a method of the simultaneous separation and determination of two kinds of antibiotics concomitantly administrated, is also useful for the determination of the metabolic time lag in the liver and the presence in bile of not only parent compounds moreover, glucuronide conjugate, metabolite and epimers could be detected. Therefore, this methodology can be usefully employed for the planning of the administration of antibiotics and the adjustment of their dosages and monitoring of their concentrations in biological fluids, especially in critical patient being treated with two or more antibiotics.

Acknowledgement

The author wishes to express deep gratitude to Prof. NORINORI HIKASA, Second Department of Surgery, Faculty of Medicine, Kyoto University, for his overall instruction, and to Dr. HIROSHI TANIMURA, Second Department of Surgery, Faculty of Medicine, Kyoto University, for his kind guidance and active participation in this experiment.

A part of the abstract of this paper was presented at the 30th Annual Meeting of the Japan Society of Chemotherapy in Tokyo, in 1982, and at the 30th Annual Meeting of the Western Japan Society of Chemotherapy in Nagoya, in 1982.

Reference

- 1) Abdel Aziz FT, Hirom PC, et al: The biliary excretion of anions of molecular weight 300-800 in the rat, guinea pig and rabbit. *Biochem J* **125**: 25-26, 1971.
- 2) Abou-El-Makarem MM, Millburn P, et al: Biliary excretion of [¹⁴C] succinylsulphathiazole in the rat and rabbits. *Biochem J* **105**: 1295-1299, 1967.
- 3) Abou-El-Makarem MM, Millburn P, et al: Biliary excretion of foreign compounds. Species differences in biliary excretion. *Biochem J* **105**: 1289-1293, 1967.
- 4) Acocella G, et al: Biliary excretion of antibiotics in man. *Gut* **9**: 536-545, 1968.
- 5) Alrawi ZH, Tabaqchali S: Fluoremetric determination of cefuroxime in body fluids. *Antimicrob Agents Chemother* **20**(1): 25-29, 1981.
- 6) Anhalt JP: Clinical antibiotic assay by HPLC. In biological, biomedical application of liquid chromatography (HAWK GL.ed.), Vol. II P. 1-16, Marcel Dekker, New York, 1980.
- 7) Aziz NS, Gambertoglio JG, et al: Pharmacokinetics of cefamandole using a HPLC assay. *J Pharmacobiopharm* **6**: 153-164, 1978.
- 8) Barza M, Brown RB, et al: Relation between lipophilicity and pharmacological behavior of minomycin, doxycycline, tetracycline, and oxytetracycline in dogs. *Antimicrob Agents Chemother* **8**: 713-620, 1975.
- 9) Barza M: Principles of tissue penetration of antibiotics. *Antimicrob Chemother* **8** (Suppl): 7-28, 1981.
- 10) Benner EJ: Two-hour assay for content of penicillins and cephalosporins in serum. *Antimicrob Agents Chemother* 201-204, 1970.
- 11) Bourgault AM, Douglas M, et al: Clinical characteristics of anaerobic bactibilia. *Arch Intern Med* **139**:

- 1346-1349, 1979.
- 12) Broughton A, Strong JE: Radioimmunoassay of iodinated gentamicin. *Clin Chim Acta* **66**: 125-129, 1976.
 - 13) Broughton A, Strong JE, et al: Radioimmunoassay of sisomicin. *Antimicrob Agents Chemother* **9**: 247-250, 1976.
 - 14) Buhs RP, Maxim TE, et al: Analysis of cefoxitin, cephalothin and their deacetylated metabolites in human urine by high performance liquid chromatography. *J Chromatogr* **99**: 609-618, 1974.
 - 15) Carroll MA, White ER, et al: The determination of cephalixin and cephadrine by reverse phase chromatography. *J Antibiot* **30**: 397-403, 1977.
 - 16) Cooper MJ, Anders MW, et al: Ion-pair extraction and highspeed chromatography of cephalothin and deacetyl-cephalothin in human serum and urine. *Drug Metab Dispos* **1**: 659-662, 1973.
 - 17) Craig WA, Kunin CM: Significance of serum protein and tissue binding of antimicrobial agents. *Ann Rev Med* **27**: 287-300, 1976.
 - 18) Edberg SC, Chu A: Determining antibiotic levels in the blood. *Am J Med Techn* **41**: 99-105, 1975.
 - 19) Ford JH, Prescott GG, et al: Colorimetric determination of erythromycin. *Anal Chem* **25**: 1195-1197, 1953.
 - 20) Ghebre SI, Hem SL, et al: Separation of penicillin and its major degradation products by ion-pair reverse-phase high pressure liquid chromatography. *J Pharm Sci* **71**(3): 351-353, 1982.
 - 21) Gilbertson TJ, Stryd RP: Radioimmunoassay for clindamycin. *Clin Chem* **22**: 828-831, 1976.
 - 22) Gundert-Remy U, Forster D, et al: Kinetics of mezlocillin in patients with biliary T-tube drainage. *Antimicrob chemother* **9** (supp A): 65-75, 1982.
 - 23) Haas MJ, Davies J: Enzymatic acetylation as a means of determining serum aminoglycoside concentrations. *Antimicrob Agents Chemother* **4**: 497-499, 1973.
 - 24) Habig WH, Pabst MJ, et al: The identify of Glutathione S-transferase B with ligandin, a major protein of liver. *Proc Nat Acad Sci USA* **71**(10): 3879-3882, 1974.
 - 25) Hayashi Y: High-performance liquid chromatographic microassay for cefradine in biological fluids. *Jpn J Antibiot* **34**(3): 204-210, 1981.
 - 26) Hirom PC, Millburn RL, et al: Species variation in the threshold molecular weight factor for the biliary excretion of organic anions. *Biochem J* **129**: 1071-1077, 1972.
 - 27) Hirom PC, Millburn P: Bile and urine as complementary pathways for the excretion of foreign organic compounds. *Xenobiotica* **6**: 55-64, 1976.
 - 28) Huang WF, Tanimura H, et al: The simultaneous determination of two new cephalosporins in bile and serum by high performance liquid chromatography. *Proc 30th Ann Meeting Jpn Soc Chemother Tokyo*, p. 131, 1982.
 - 29) Huang WF, Tanimura H, et al: A comparative study of biliary excretion of antibiotics after simultaneous administration by HPLC analysis. *Proc 30th Ann Meeting West Jpn Soc Chemother Nagoya*, p. 55, 1982.
 - 30) John GR, Kent BC, et al: Cefaclor pharmacokinetic parameters: serum concentrations determined by a new high-performance liquid chromatographic technique. *Antimicrob Agents Chemother*: **21**(1) 170-172, 1982.
 - 31) Kakemi K, Arita T, et al: Absorption and excretion of drugs. IV. Determination of chloramphenicol in blood. *Yakugaku Zasshi* **82**: 342-345, 1963.
 - 32) Kirsch R, Fleischner G, et al: Structure and functional studies of ligandin, a major renal organic anion-binding protein. *J Clin Invest* **55**: 1009-1019, 1975.
 - 33) Kornuth ML, Monson RA, et al: Binding of antibiotics to a soluble protein from rat liver. *J Inf Dis* **129**(5): 552-558, 1974.
 - 34) Lecaillon JB, Hirtz JL, et al: Pharmacokinetic comparison of cefroxadin (CGP 900) and cephalixin by simultaneous administration to humans. *Antimicrob Agents Chemother* **18**(4): 656-660, 1980.
 - 35) Lecaillon JB, Rouan MC, et al: Determination of cefsulodin, cefotiam, cephalixin, cefotaxime, desacetyl-cefotaxime, cefuroxime and cefroxadin in plasma and urine by highperformance liquid chromatography. *J Chromatogr* **228**: 257-267, 1982.
 - 36) Lee TL, Darconte L, et al: High-pressure liquid chromatographic determination of amoxicillin in urine. *J Pharm Sci* **68**(4): 454-458, 1979.
 - 37) Lesar TS, Zaske DE: Antibiotics and hepatic disease. *Med Clin Nor Am* **66**(1): 257-265, 1982.
 - 38) Luthy R, Blaser J, et al: Comparative multiple-dose pharmacokinetics of cefotaxime, moxalactam, and ceftazidime. *Antimicrob Agents Chemother* **20**(5): 567-575, 1981.
 - 39) Luthy R, Blaser J, et al: Human pharmacokinetic of ceftazidime in comparison to moxalactam and cefotaxime-abstract. *Antimicrob chemother* **8**(Suppl B): 273-276, 1981.
 - 40) Liver M: Improved fluorimetric determination of tetracyclines. *Biochem Med* **6**: 216-222, 1972.

- 41) Levine WG: Biliary excretion of drugs and other xenobiotics. *Ann Rev Pharmacol Toxicol* **18**: 81-96, 1978.
- 42) Marti MC, Farguet C, et al: Pharmacokinetics and biliary excretion cefoperazone in patients with bile duct drainage. *Inf* **9**(Suppl-1): 34-36, 1981.
- 43) Martinez OV, Liev JU, et al: Biliary excretion of moxalactam. *Antimicrob Agents Chemother* **20**(2): 231-234, 1981.
- 44) Miller JM, Tucker E: Use of HPLC for multicomponent serum analysis initial experiences in a hospital laboratory. *Am Lab* **11**(1): 17-34, 1979.
- 45) Miner DJ, Coleman DL, et al: Determination of moxalactam in human body fluids by liquid chromatographic and microbiological methods. *Antimicrob Agents Chemother* **20**(2): 252-257, 1981.
- 46) Nahata MC, Pharm D, et al: Moxalactam epimer kinetics in children. *Clin Pharmacol Ther* **31**(4): 528-532, 1982.
- 47) Nakagawa T, Haginaka J, et al: High-speed liquid chromatographic determination of cephalixin in human plasma and urine. *J Antibiot (Tokyo)* **31**: 769-775, 1978.
- 48) Nakagawa T, Haginaka J, et al: Direct high-speed liquid chromatographic determination of cephalixin in urine. *J Chromatogr* **147**: 509-512, 1978.
- 49) Nilsson Ehle I: High-pressure liquid chromatography as a tool for determination of antibiotics in biological fluids. *Acta Path Microbiol Scand Sect B (Suppl)* **259**: 61-66, 1977.
- 50) Nilsson Ehle I, Nilsson Ehle P: Liquid chromatographic assay of cefuroxime in serum. *Clin Chem* **24**: 365-365, 1978.
- 51) Nilsson Ehle I, Yoshikawa TT, et al: Quantitation of antibiotics by high-pressure liquid chromatography: Cephalothin. *Antimicrob Agents Chemother* **13**: 221-227, 1978.
- 52) Nishida M, Murakawa T, et al: A chromatographic assay for the mixture of aminobenzyl-penicillin and methylchlorophenylisoxazolyl-penicillin. *Chemother (Tokyo)* **17**: 1973-1976, 1969.
- 53) Nishiya H, Komatsu T, et al: Binding of antibiotics to human liver glutathione S-transferase. *Jpn J Exp Med* **51**(6): 355-362, 1981.
- 54) Polk RE, Kline BJ, et al: Cefazolin and moxalactam pharmacokinetics after simultaneous intravenous infusion. *Antimicrob Agents Chemother* **20**(5): 576-576, 1981.
- 55) Ram MD, Watanatittan S: Cephalothin levels in human bile. *Arch Surg* **108**: 187-189, 1974.
- 56) Ratzan KR, Baker HB, et al: Excretion of cefamandole, cefazolin and cephalothin into T-tube bile. *Antimicrob Agents Chemother* **13**(6): 985-987, 1978.
- 57) Rollins DE, Klaassen CD: Biliary excretion of drugs in man. *Clinic Pharmacol* **4**: 368-379, 1979.
- 58) Roth W, Beschke K, et al: A new chromatograph for pharmacokinetic drug monitoring by direct injection of body fluids. *J Chromatogr* **222**: 13-22, 1981.
- 59) Ryrfeldt A: Biliary excretion of penicillins in the rat. *J Pharm Pharmacol* **23**: 463-464, 1971.
- 60) Saito S, Yoshida M: Biliary tract infections and antibiotics, concentration of cefotiam in gallbladder tissue and bile. *Jpn J Antibiot* **35**(4): 1057-1063, 1982.
- 61) Salto F: Separation of penicillin and cephalosporin diastereoisomers by reversed-phase high-performance liquid chromatography. *J Chromatogr* **161**: 385, 1978.
- 62) Shimada K: Biliary tract infection. *Jpn J Clin Med* **39**: 1398-1399, 1981.
- 63) Schoenfield LJ: Biliary excretion of antibiotics. *N Engl J Med* **284**: 1213-1214, 1971.
- 64) Shimizu K, Watanabe Y, et al: Studies on protein binding of antibiotics. V. Effect of the binding of drug to 100,000 G supernatant fluid of human liver homogenates on urinary excretion. *J Antibiot* **35**: 1610-1516, 1982.
- 65) Shimizu T, et al: The experimental and clinical study of SN-401 in surgical field. *Proc 30th Ann Meeting Jpn Soc Chemother* p. 194-195, 1982.
- 66) Seikine M, Sasahara K, et al: High-performance liquid chromatographic method for determination of cefmetazole in human serum. *Antimicrob Agents Chemother* **21**(5): 740-743, 1982.
- 67) Tanimura H, et al: Chemotherapy of peritonitis (II) with special reference on the clinical effects of ceftizoxime. *Chemother (Tokyo)* **28**(Suppl-5): 553-542, 1980.
- 68) Tanimura H, et al: Chemotherapy on the biliary tract infection. (XIII) Miloxacin, A novel chemotherapeutic agent, its excretion into bile and clinical effect on the biliary tract infection. *Jpn J Antibiot* **34**(9): 1335-1351, 1981.
- 69) Tanimura H, Saito T, et al: Chemotherapy of biliary tract infection. *J Med Pharm* **5**(1): 35-40, 1981.
- 70) Tanimura H, Takahashi H, et al: Biliary tract infection and organ failure. *Clin Bact* **9**: 281-287, 1982.
- 71) Tanimura H: Biliary tract infection. *Jpn J Clin Exp Med* **59**(10): 3238-3244, 1982.

- 72) Tanimura H, et al: Chemotherapy of biliary tract infection (XII) with special reference on biliary excretion, tissue concentration in gallbladder and clinical effects in patients treated with Cefotetan. *Chemother (Tokyo)* **30**(Suppl-1): 976-816, 1982.
- 73) Tanimura H, et al: Chemotherapy on the biliary tract infection (XV) with special reference on biliary excretion, tissue concentration in gallbladder and clinical effects in patients treated with Cefpiramide. *Chemother (Tokyo)* **31**(Suppl): 1983, (in press).
- 74) Tanimura H, et al: Chemotherapy on the biliary tract infection (XVI) with special reference on biliary excretion of T-1982 and its stability in bile. *Chemother (Tokyo)* **30** (Suppl-3): 175-189, 1982.
- 75) Tanimura H, et al: Chemotherapy on the biliary tract infection (XVIII) with special reference on biliary excretion, tissue concentration in gallbladder and excretion into peritoneum and clinical effects in patients treated with Cefazidime. *Chemother (Tokyo)* **31**: 1983, (in press).
- 76) Tanimura H, et al: Chemotherapy on the biliary tract infection (XX) with special reference on the tissue concentration in gallbladder and biliary excretion and clinical effects in patients treated with Ceftriaxone. *Chemother (Tokyo)* **31**, 1983, (in press).
- 77) Tserng KY, Wagner JG: Fluorimetric determination of erythromycin propionate in whole blood plasma and correlation of results with microbiological assay. *Anal Chem* **48**: 348-353, 1976.
- 78) Tune BM: Effect of organic acid transport inhibitors on renal cortical uptake and proximal tubular toxicity of cephaloridine. *J Pharmacol Exp Ther* **181**: 250-256, 1972.
- 79) White ER, Carroll MA, et al: Reverse phase high speed liquid chromatography of antibiotics. *J Antibiot* **28**: 205-214, 1975.
- 80) White ER, Carroll MA, et al: Reverse phase high-speed liquid chromatography of antibiotics. II. Use of high efficiency small particle columns. *J Antibiot* **30**: 811-818, 1977.
- 81) White ER, Zarembo MA: Reverse phase high-speed liquid chromatography of antibiotics. III. Use of ultra high performance columns and ion-pairing techniques. *J Antibiot* **34**: 836-844, 1981.
- 82) White LD, Holt HA, et al: Separation and assay of cefotaxime (HR-756) and its metabolites in serum urine and bile. Proceedings of the 11th international congress of chemotherapy and the 19th interscience conference of antimicrobial agents and chemotherapy. Am Soc Microbio Washington, DC, p. 153, 1980.
- 83) Wise R, Will PJ, et al: Epimers of moxalactam: in vitro comparison of activity and stability. *Antimicrob Agents Chemother* **20**: 30-32, 1981.
- 84) Wold JS, Turnispeed SA: Determination of cephaloridine in serum and tissue by high-performance liquid chromatography. *J Chromatogr* **136**: 170-173, 1977.
- 85) Wold J: Rapid analysis of cefazolin in serum by highpressure liquid chromatography. *Antimicrob Agents Chemother* **11**: 105-109, 1977.
- 86) Wold JS, Turnispeed SA: The simultaneous quantitative determination of cephalothin and cefazolin in serum by high pressure liquid chromatography. *Clin Chim Acta* **78**: 203-207, 1977.
- 87) Wright WE, Line VD, et al: Biliary excretion of cephalosporins in rats: influence of molecular weight. *Antimicrob Agents Chemother* **17**(5): 842-846, 1980.
- 88) Yamada H, Ichihashi K, et al: Plasma protein binding and urinary excretion of R- and S-epimer of an aryl-malnylamino 1-oxacephem I: in humans. *J Pharm Sci* **70**: 112-113, 1981.
- 89) Yamaoka K, Narita S, et al: High-performance liquid chromatographic analysis of sulbenicillin and carbenicillin in human urine. *J Chromatogr* **168**: 187-193, 1979.
- 90) Yoshikawa TT, Maitra SK, et al: High-pressure liquid chromatography for quantitation of antimicrobial agents. *Rev Inf Dis* **2**(2): 169-181, 1980.

和文抄録

同時投与同時分離定量法による抗生物質の
新しい比較試験法

京都大学医学部外科学教室第二講座(指導:日笠頼則教授)

黄 文 芳

従来、抗生物質の体液中濃度測定は bioassay 法で行われてきたため、その結果が判明するまでには病態が変化してしまい、臨床の実際には役立たなかった。また、正常動物や健康人における体内分布、代謝に関する資料では、多くの機能障害を伴っている感染症の臨床には適応できない。例えば、胆道感染症では、患者の肝機能や黄疸の状態などの背景因子が症例毎に異なるため、数症例における成績の算術的平均値を指標にするのではなく、著者らは、同一症例における2剤以上の crossover 投与法による比較試験が有用であることを強調してきた。しかし、それでもなお何れの薬剤を先に投与するか、あるいは、試験日の胆汁排泄量の日差変動などの問題点を残していた。著者は、従来の bioassay 法では不可能であった同系統の薬剤が混在している胆汁試料における抗生物質の測定濃度には高速液体クロマトグラフィー(HPLC)法が有用であることに注目し、また最近、嫌気性菌の分離固定技術の向上から、複数菌感染症が急増し、2ないし3剤以上の抗生物質併用療法が必要となり、かかる症例における体液中抗生物質濃度の測定やモニタリング法として応用できることを目的として、2種類の Cephalosporin または Cepharmycin 系薬剤を同時に投与し、全く同一条件下で比較できる HPLC 法を開発し、ラット及びヒトに実験を行い、以下の結果を得た。

1) 2剤またはそれ以上の Cephalosporin または Cepharmycin を含む血清、胆汁、及び肝ホモジネート上清における、それぞれの抗生物質の同時分離定量が可能な HPLC 条件を開発した。

2) 体重 300 g の SD 系雄ラット30匹を Ceftazidime (CAZ), Ceftizoxime (CZX) 20 mg/kg 単独群と各々 20 mg/kg 同時投与群に分け、投与20, 50, 80分後の血清と肝臓を採取し、胆汁は0-20, 21-50, 51-80分間の3分画に分けて採取した。採取した試料を先に開発した独自の HPLC 2剤同時分離法で測定した結果、薬剤間の胆汁中移行における競合は認められず、胆汁中分泌速度、胆汁酸濃度の影響、他の電解質、胆汁中

蛋白量などの影響を完全に除外できることが判明した。

この方法を実験動物に応用し、それら両薬剤の血清濃度曲線が類似している際にも、両薬剤の肝組織内濃度の上昇に差異があり、それが胆汁中濃度パターンの変化の主因となり、2剤間に明らかな胆汁中移行の相違を生ずるという一連の代謝過程を、同一個体において証明した。

3) 総胆管ドレナージを施行中の6症例において、Cephalosporin または Cepharmycin 2剤投与時における血中濃度、胆汁中濃度を測定し、先の動物実験で立証した如く、血中濃度の推移が類似した薬剤でも、胆汁中移行パターンが著しく異なるものもあることを、従来の bioassay による日を変えた crossover 比較試験法では除外できなかった胆汁酸をはじめとする胆汁成分の腸肝循環の破綻をきたすこともなく、また、胆汁採取日による胆汁流出量の相違などにも影響されることなく、証明し得た。

また、一般に黄疸時には非黄疸時よりも抗生物質の胆汁中濃度および総排泄量の減少をみることが多いがこの問題に関して、同一症例に黄疸時と非黄疸時に、CZX 及び CAZ を例として、同時投与比較試験を行った結果、一方、(CZX) は黄疸の有無に拘わらず、同じ胆汁中移行パターンを示したのに対し、他方、(CAZ) は非黄疸時には投与後4~5時間に最高胆汁中濃度を示したが、黄疸時には胆汁中濃度のピークが1~2時間後に存在する前者と同じ移行パターンを示した。この事実から、ある種の抗生物質とくに Cephalosporin 系薬剤 (CAZ) の胆汁中移行は胆汁酸量により支配されている可能性が示唆された。

以上、HPLC 法を用いる著者の開発した2剤同時投与同時分離定量法には、その他、薬剤本体(未変化体)のみならず、グルクロン酸抱合体や代謝体ないし異性体の胆汁中排泄の有無および肝における代謝時間の“ずれ”の測定にも有用であり、今後の抗生物質投与計画の立案および投与経路、投与量の調整などのモニタリング法として極めて役立つものといえよう。